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Annealing-Induced Changes in Double-Brush Langmuir–Blodgett Films of α -Helical Diblock Copolypeptides

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The effect of annealing on the structure and the helix orientation in Langmuir–Blodgett (LB) monolayers of diblock copolymers (PLGA-*b*-PMLGSLGs) of poly(α -L-glutamic acid) (PLGA) and poly(γ -methyl-L-glutamate-*ran*- γ -stearyl-L-glutamate) with 30 mol % of stearyl substituents (PMLGSLG) with unidirectional helix orientation deposited on hydrophilic silicon substrates was characterized by means of small-angle X-ray reflectivity, transmission Fourier transform infrared spectroscopy, and atomic force microscopy. Upon annealing at 100 °C for 24 h, the α -helices became less tilted toward the substrate surface normal. Surface area shrinkage accompanied the change in tilt, indicated by an increase in both film thickness and electron density, resulting in more compact and uniform films. The enhancement of the helix orientation by thermal annealing was greater for the PMLGSLG block and for the diblock copolymers with the shorter block lengths. For these diblock copolymers, annealing resulted in postorientation of the PMLGSLG block helices almost perpendicular to the substrate surface. This effect originates from a considerable increase in intermolecular packing of the PLGA block caused by hydrogen bonds between the carboxylic groups upon annealing, as well as the high mobility of the PMLGSLG block helices for rearrangement favored by the melted side chain mantle at elevated temperatures.

Introduction

Thin films of oriented α -helical polypeptides have attracted considerable attention due to their remarkable electro-optical properties generated by unidirectionally aligned helix macrodipoles.^{1,2} Studies of self-assembled polypeptide systems have shown potential applications in chemical biology, opto-electronics, and biosensors.^{3–6}

Among synthetic polypeptides, hairy-rod polyglutamates have been widely studied because of their surface activity and liquid crystalline behavior.^{7–10} Their structures are useful for many optical and photoconductive applications.^{11–13} Therefore, highly ordered monolayers of hairy-rod polyglutamates with unidirectional helix orientation show promising properties for thin film applications requiring incorporation and orientation of bio- and optical-molecules. The surface-grafting-from technique has been

used to orient the chains of hairy-rod polyglutamates at solid surfaces.^{14,15} We have studied 4–5 nm thick double-brush monolayer films containing segments of poly(γ -methyl-L-glutamate-*ran*- γ -stearyl-L-glutamate) with 30 mol % of stearyl substituents (PMLGSLG), unidirectionally oriented by the Langmuir–Blodgett (LB) technique, using poly(α -L-glutamic acid)-*b*-poly(γ -methyl-L-glutamate-*ran*- γ -stearyl-L-glutamate) (PLGA-*b*-PMLGSLG) diblock copolymers.^{16,17} The use of the LB technique in film fabrication overcomes the disadvantage of the difficulty in analyzing grafted polymers and has the advantage to readily incorporate and orient guest molecules into the films.

Notably, for the PLGA-*b*-PMLGSLG diblock copolymer system, we found that the helix length strongly affects the helix tilt order of the polypeptide brush.¹⁷ This effect has been tentatively attributed to the dipolar interactions of the off-axis components of the unscreened peptide dipoles between parallel aligned α -helices. As a result, the average helix tilt angle between the helix axis and the surface normal increases with increasing the helix length in order to lower the interaction energy of the oriented dipoles in a smectic C-like phase.¹⁷ For example, the average tilt angle has been reported to be 48–66° for surface-grafted PMLGSLG films.^{14,15} For LB films of the PLGA-*b*-PMLGSLG diblock copolymers, where the alkyl side chains surround the PMLGSLG block segments, the smallest average helix tilt angle obtained for the PMLGSLG block was 29°.¹⁷

Several film treatment methods to induce permanent perpendicular helix orientation in surface-grafted polypeptide films have been reported, such as film swelling in *n*-hexadecane,¹⁴ by means

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of solvent-quenching¹⁸ or interhelical cross-linking.¹⁵ In order to further manipulate the helix orientation in the LB monolayers of PLGA-*b*-PMLGSLGs, we combined advantages of the PLGA block containing a side chain carboxylic acid group capable of forming hydrogen bonds and the mobility of the side chain mantle of the PMLGSLG block. By means of annealing, interchain hydrogen bonding interaction is enhanced between the carboxylic groups of the PLGA blocks and changes in the helix packing of one block are expected to alter the orientation of the other.

This paper reports on the effect of annealing on the structure and helix orientation of the LB monolayers of the PLGA-*b*-PMLGSLG diblock copolymers, studied by small-angle X-ray reflectivity (XRR), transmission Fourier transform infrared (FT-IR) spectroscopy, and atomic force microscopy (AFM).

Experimental Section

Materials. A detailed description of the synthesis of PLGA-*b*-PMLGSLGs can be found elsewhere.¹⁶ In short, α -helical PLGA-*b*-PMLGSLG was synthesized via a diblock copolymer precursor consisting of poly(γ -*tert*-butyl-L-glutamate) (PtBuLG) and PMLGSLG (with 30 mol % of stearyl substituents), with the *tert*-butyl group as a mild acid-labile, protecting group for the carboxylic acid. PtBuLG-*b*-PMLGSLG was synthesized employing the primary amine-initiated stepwise ring-opening polymerization of α -amino acid *N*-carboxyanhydrides (NCAs) in chloroform at 0 °C. The molecular weights and polydispersity indexes (PDIs) of the PtBuLG-*b*-PMLGSLGs were characterized by ¹H NMR (CDCl₃) and gel permeation chromatography (tetrahydrofuran eluent, polystyrene standard, universal calibration).^{16,19} The *tert*-butyl group was removed using trifluoroacetic acid (TFA). PMLGSLG with 30 mol % of stearyl substituents (DP = 118) was prepared by NCA random copolymerization in chloroform at 0 °C using triethylamine as initiator. All the copolymers synthesized were in the α -helical conformation, as characterized by FT-IR.¹⁶

PLGA-*b*-PMLGSLG diblock copolymers are abbreviated as CoPo-*m*-*n*, where *m* and *n* denote the degrees of polymerization (DPs) of the PLGA and PMLGSLG blocks, respectively. Three diblock copolymers were used for the study: CoPo-60-19 (PDI_{PLGA block} = 1.16, PDI_{CoPo-60-19} = 1.32), CoPo-37-24 (PDI_{PLGA block} = 1.25, PDI_{CoPo-37-24} = 1.30), and CoPo-59-82 (PDI_{PLGA block} = 1.16, PDI_{CoPo-59-82} = 1.15).

Film Preparation and Annealing. The substrate cleaning procedure was described elsewhere.¹⁶ The LB film transfer was performed using a home-modified computer-controlled Lauda Filmbalance (FW2) instrument, with an accuracy of 0.05 mN/m. The water used for the subphase was purified by reverse osmosis and subsequently through a Milli-Q filtration system. PMLGSLG was spread from a chloroform solution at a concentration of 0.6 mg/mL. PLGA-*b*-PMLGSLGs were spread from *N*-methylpyrrolidone (NMP) (Acros, 99.5%)/chloroform (Lab-Scan, 99.5%) (3/7, v/v) solutions with 1–3% (v/v) acetic acid (Acros, 99.5%) added, at a concentration of 0.4–0.6 mg/mL. The small amount of acetic acid was added to improve the solubility of the copolymers in the solvent mixture and did not noticeably influence the conformation of the copolymers as well as the monolayer properties. Polymer solutions were spread dropwise on the water surface using a microsyringe. The monolayer was allowed for 15–30 min to equilibrate, during which the spreading solvent could either evaporate (chloroform) or mix with the subphase (NMP, acetic acid). Under the spreading and compression conditions used, the monolayers of PLGA-*b*-PMLGSLGs were found to consist of molecules that are in a predominantly α -helix structure.¹⁶ Vertical deposition of a PLGA-*b*-PMLGSLG monolayer onto a chemically

cleaned silicon substrate was done at a subphase temperature of 20 \pm 0.1 °C, at down- and upstroke speeds of 100 and 10 mm min⁻¹, respectively, for CoPo-59-82 and at down- and upstroke speeds of 100 and 1 mm min⁻¹ for the other diblock copolymers. Monolayers of PLGA-*b*-PMLGSLGs were deposited onto both sides of hydrophilic substrates during the upward stroke. Transfer ratios were around unity. A multilayer film of PMLGSLG can be deposited onto a hydrophobic substrate by Y-type transfer, at down- and upstroke speeds of 3 mm min⁻¹.

The LB monolayers of three diblock copolymers with different block lengths, CoPo-60-19, CoPo-37-24, and CoPo-59-82, deposited on both sides of double-sided polished silicon substrates at transfer pressures of 40, 45, and 35 mN/m, respectively, were used for investigation of the annealing effect. A multilayer LB film of PMLGSLG (DP = 118) was deposited at 20 mN/m on a hydrophobized double-sided polished silicon substrate (six layers on each side of the substrate). In annealing experiments, the films were placed in an oven at 100 °C for a period of time, cooled down to room temperature, and characterized by AFM, transmission FT-IR, and small-angle XRR measurements.

Small-Angle X-ray Reflectivity (XRR). Small-angle XRR measurements of LB films on silicon substrates were performed in $\theta/2\theta$ geometry on a Philips X'pert materials research diffractometer (MRD) instrument, employing Cu K α radiation of 1.541 Å and with a divergence slit of 1/8 degrees, an antiscatter slit of 1/4 degrees, and a progressive receiving slit of 0.3 nm. The X-ray tube was operated at V = 40 kV and I = 40 mA. The beam was 12 \times 0.3 mm in size after passing through the divergence slit and was monochromatic, horizontally parallel, and vertically divergent. A more detailed description of the setup can be found elsewhere.²⁰ For analysis, the measured reflectivity, R , was normalized by the Fresnel reflectivity, R_F .²¹ To model the electron density distribution along the z -direction, the film was divided into slabs (boxes) with thicknesses d_i , electron densities ρ_i , and interface roughnesses σ_i between slabs i and $i + 1$, using a homemade computer program written in IDL 6.0 by Hibma et al.²² The fitting procedure was based on Paratt's algorithm²³ with the Névo–Croce formula for interface roughness.²⁴ A separate SiO₂ layer was not taken into account in the simulation, as this layer contributes insignificantly to the reflectivity of the wafer ($\rho_{\text{SiO}_2}/\rho_{\text{Si}} = 0.95$) and could not be resolved for the reflectivity curves of bare silicon wafers.²⁵ A two-slab model composed of consecutive layers of the PLGA and PMLGSLG blocks with three surface roughnesses were employed to simulate the XRR curves.^{16,17} The model parameters were varied, with constraints on the fitting parameters, until acceptable agreement between the experimental and calculated model reflectivity was achieved.^{16,17} The goodness of the least-squares fit was determined by minimization of χ^2 .

Atomic Force Microscopy (AFM). AFM measurements were carried out on a Digital Instruments MultiMode atomic force microscope equipped with a Nanoscope IIIa controller in tapping mode using silicon cantilevers (Veeco, model RTESPW). For each sample, AFM scans were performed on several surface positions to check the surface uniformity.

Transmission Fourier Transform Infrared (Transmission FT-IR). Transmission FT-IR measurements of films on double-sided polished silicon substrates were performed at a resolution of 3 cm⁻¹, under vacuum on a Bruker IFS66 V/S FT-IR spectrometer equipped with a MIR DTGS detector. A sample shuttle accessory was used for interleaved sample and background scanning. A clean silicon substrate was used as the reference. Each spectrum is an average of 40 cycles of 120 scans.

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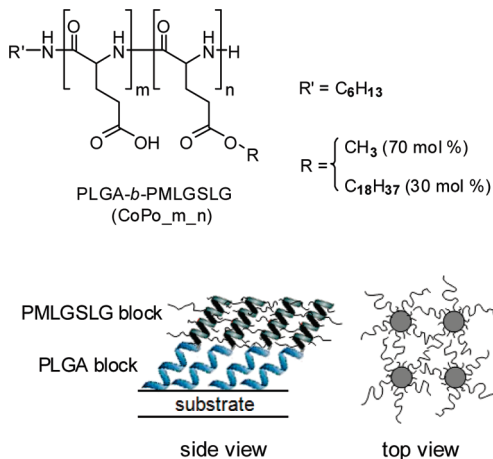
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Table 1. Best Two-Slab Fit Parameters for the XRR Curves of the PLGA-*b*-PMLGSLG LB Monolayers Transferred onto Silicon Substrates before and after Annealing^a

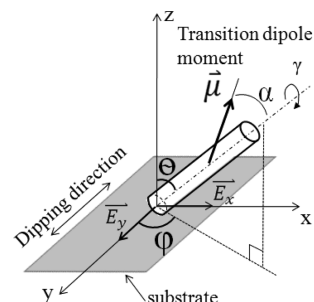
	CoPo_60_19			CoPo_37_24		CoPo_59_82	
	before annealing	after annealing for 4 h	after annealing for 24 h	before annealing	after annealing for 24 h	before annealing	after annealing for 24 h
	layer thickness (nm)						
L_1 (PLGA)	2.04 ± 0.06	2.27 ± 0.08	2.30 ± 0.08	2.20 ± 0.08	2.35 ± 0.12	1.30 ± 0.06	1.30 ± 0.07
L_2 (PMLGSLG)	2.27 ± 0.07	2.41 ± 0.08	2.71 ± 0.06	3.14 ± 0.09	3.51 ± 0.07	2.26 ± 0.07	2.41 ± 0.08
total thickness	4.31 ± 0.13	4.68 ± 0.16	5.01 ± 0.14	5.34 ± 0.17	5.86 ± 0.19	3.56 ± 0.13	3.71 ± 0.15
	electron density ρ_i ($10^3 \text{ e}^-/\text{nm}^3$)						
ρ_0 (silicon)	0.705^b	0.705^b	0.705^b	0.705^b	0.705^b	0.705^b	0.705^b
ρ_1 (PLGA)	0.478 ± 0.013	0.482 ± 0.012	0.543 ± 0.013	0.480 ± 0.015	0.546 ± 0.018	0.474 ± 0.012	0.549 ± 0.016
ρ_2 (PMLGSLG)	0.350 ± 0.009	0.365 ± 0.010	0.378 ± 0.007	0.389 ± 0.010	0.428 ± 0.012	0.368 ± 0.009	0.391 ± 0.009
	interface roughness (nm) ²⁸						
σ_{01} (silicon/PLGA)	0.63 ± 0.08^c	0.76 ± 0.09^c	0.56 ± 0.07^c	0.74 ± 0.09^c	0.88 ± 0.11^c	0.48 ± 0.04^b	0.61 ± 0.13^c
σ_{12} (PLGA/PMLGSLG)	0.76 ± 0.12	0.81 ± 0.11	1.32 ± 0.18	0.98 ± 0.25	1.70 ± 0.13	0.35 ± 0.15	0.78 ± 0.15
σ_{23} (PMLGSLG/air)	0.31 ± 0.04	0.15 ± 0.05	0.05 ± 0.03	0.16 ± 0.02	0.34 ± 0.03	0.18 ± 0.05	0.06 ± 0.05
	average tilt angles estimated from layer thicknesses (deg)						
θ_1 (PLGA)	76.9 ± 0.4	75.4 ± 0.5	75.2 ± 0.5	66.6 ± 0.9	64.9 ± 1.4	81.6 ± 0.4	81.6 ± 0.5
θ_2 (PMLGSLG)	37.2 ± 2.4	32.3 ± 3.2	18.0 ± 4.4	29.3 ± 3.1	12.8 ± 6.8	79.4 ± 0.3	78.7 ± 0.4

^a The error of a certain fit parameter was estimated from the change in the parameter in order to increase the χ^2 value of the fit by 10%. The errors of the tilt angles were derived from those of the corresponding layer thicknesses. ^b Values kept fixed for the curve fits. ^c σ_{01} was first fixed at 0.48 ± 0.04 nm, the average roughness of bare silicon wafers, and the other parameters were varied until the model adequately simulated the experimental reflectivity ($\chi^2 < 10^{-3}$). Then σ_{01} was adjusted to obtain a good curve fit, after which further refinement of the other fitted ρ and L parameters for χ^2 minimization only resulted in slight changes in these parameters by less than 2% of their values (see the Supporting Information for a demonstration of how σ_{01} was adjusted).

Scheme 1. Chemical Structure of PLGA-*b*-PMLGSLG and Simple Schematic Representation of the Double-Brush Structure of the PLGA-*b*-PMLGSLG LB Monolayer



Scheme 2. Helix Coordinate System



θ values of 67–82° and 29–79°, estimated from the layer thicknesses, were obtained for the PLGA block and PMLGSLG block in the PLGA-*b*-PMLGSLG LB monolayers, respectively, depending on the block lengths (see Table 1). The PLGA-*b*-PMLGSLG diblock copolymers were found to be thermally stable up to 225 °C, with the α -helix structure preserved up to 150 °C (see the Supporting Information).

The XRR curves of the PLGA-*b*-PMLGSLG LB monolayers after annealing at 100 °C show an increase in both layer thicknesses and electron densities (Figures 1–3 and Table 1). The two-slab models simulating the PLGA-*b*-PMLGSLG double-brush structure give good fits of all the X-ray diffractograms. AFM analysis of the films before and after annealing shows a homogeneous and uniform structure (Figure 4). These results suggest that upon annealing the films shrink from the edge to the center. As a result, the structure of the films becomes more packed. The average surface roughnesses determined by AFM and XRR measurements indicate that the film surface remained smooth after annealing. The increase in thickness and electron density associated

Results and Discussion

In our previous study,^{16,17,26} the LB monolayers of the PLGA-*b*-PMLGSLG diblock copolymers after deposition were determined to be uniform and exhibit the α -helical double-brush structure (Scheme 1) with an average helix tilt angle (θ , Scheme 2) and a preferential azimuthal helix orientation (φ , Scheme 2). The average helix tilt angle between the helix axis and the surface normal (θ) can be detected both by transmission FT-IR and from the brush thickness determined by XRR.¹⁷ Estimation of the average helix tilt angle (θ) from the brush layer thickness (L) and the degree of polymerization (DP) follows the relation:

$$\cos \theta = \frac{L}{0.15\text{DP}}, \quad \theta < 90^\circ \quad (1)$$

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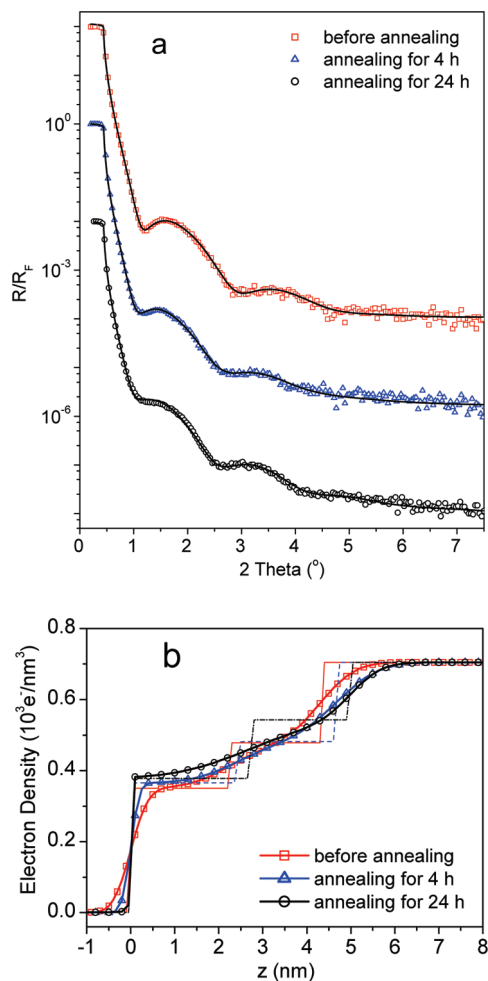


Figure 1. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo_{60_19} transferred onto silicon substrates at 40 mN/m before and after annealing; the dots represent the experimental data, and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) Electron density profiles corresponding to the curve fits (smooth curves); for clarity, the same electron density profiles are shown assuming all interface roughnesses to be zero (steplike curves).

with film shrinkage occurs gradually, indicated by a gradual change in the XRR pattern with annealing time up to 24 h (demonstrated for CoPo_{60_19} in Figure 1 and Table 1). The increase in thickness observed for both the PLGA and PMLGSLG slabs of the double-brush monolayers suggests a decrease in the average helix tilt angles. The estimated θ values for the two blocks in the PLGA-*b*-PMLGSLG LB films are shown in Table 1. The change in the helix orientation is dominant for the PMLGSLG block layer and for the two diblock copolymers with the shorter block lengths (CoPo_{60_19} and CoPo_{37_24}). For these diblock copolymers, annealing resulted in an orientation of the PMLGSLG block segments almost perpendicular to the substrate surface.

More evidence for the change in the helix orientation in the annealed films was observed by transmission FT-IR. The transition dipole moments of the amide I (1653 cm⁻¹) and amide A (3290 cm⁻¹) vibrations are oriented nearly parallel to the helix axis, while that of the amide II (1546 cm⁻¹) is nearly perpendicular to the helix axis.¹⁴ Thus, the decrease in the amide I/amide II and amide A/amide II band area ratios shown for the annealed films in Figure 5 clearly indicates a decrease in the average helix tilt angles. It is noteworthy that the oscillator strength of the amide II mode is significantly lower than that of the amide I mode²⁷ and, hence, the

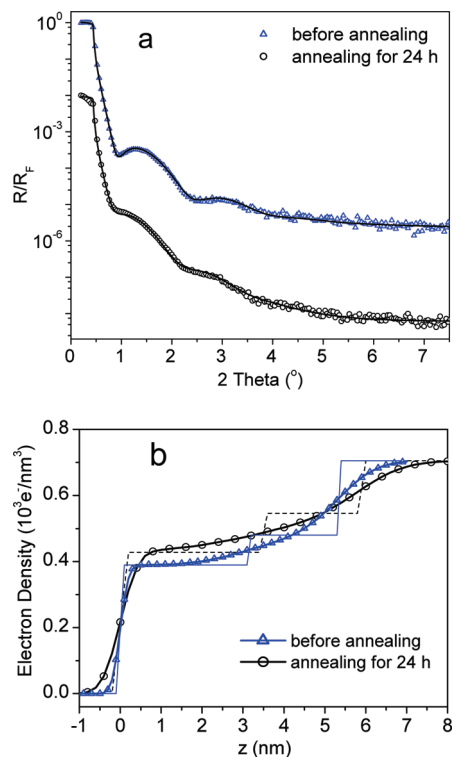


Figure 2. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo_{37_24} transferred onto silicon substrates at 45 mN/m before and after annealing; the dots represent the experimental data, and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) Electron density profiles corresponding to the curve fits (smooth curves); for clarity, the same electron density profiles are shown assuming all interface roughnesses to be zero (steplike curves).

amide II band is much less sensitive to tilt angle change than its amide I counterpart. Besides, the amide I to amide II oscillator strength ratio of PMLGSLG was indicated to be much higher than that of PLGA.¹⁷ Therefore, the pronounced change in intensity of the amide I band compared to the insignificant absorbance change of the amide II band as shown in Figure 5 may suggest a greater change in the helix tilt angle for the PMLGSLG block than for the PLGA block. The results of deconvolution of the spectra and calculation of the average helix tilt angle are in agreement with those from the XRR measurements. In addition, no change in the tilt angle of the annealed samples was detected upon storage under atmospheric conditions at room temperature for many months, indicating that the effect was irreversible upon cooling.

The change in the LB films of the PLGA-*b*-PMLGSLG diblock copolymers might likely originate from the removal of a considerable amount of water molecules bound to the PLGA block upon drying at 100 °C. This leads to stronger intermolecular

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(28) In LB monolayer deposition, initially a thin layer of water exists between the transferred monolayer and the hydrophilic substrate surface. Due to the strong hydrophilicity of the PLGA block, physically a substantial amount of water may be trapped in the film. To some extent, the slightly higher substrate/PLGA slab interface surface roughness than that of bare substrates could arise from the evaporation of water after film transfer. It is also noteworthy that in Langmuir monolayers of PLGA-*b*-PMLGSLGs while the hydrophobic PMLGSLG block is oriented with a tilt angle, the PLGA block forms a brush with the chains dispersed in the water subphase (see ref 17). In transferred monolayers, the tilting and arrangement of the PLGA block rod segments in order to properly fill space and conform to the asperities of the solid substrate are determined by many factors, including the orientation of the PMLGSLG block, the molecular packing of the layer, as well as the interactions of the PLGA block with the substrate, and can certainly influence the interface roughnesses.

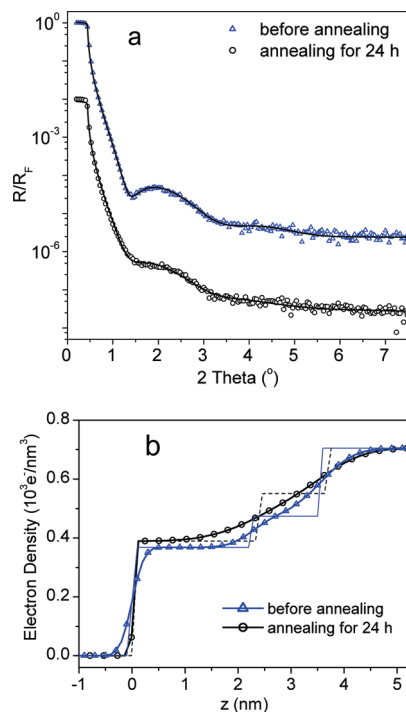


Figure 3. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo_{59_82} transferred onto silicon substrates at 35 mN/m before and after annealing; the dots represent the experimental data, and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) Electron density profiles corresponding to the curve fits (smooth curves); for clarity, the same electron density profiles are shown assuming all interface roughnesses to be zero (steplike curves).

interactions between the PLGA block segments arising from hydrogen bond formation between the carboxylic groups. The FT-IR C=O stretching vibration of the carboxylic groups in the PLGA-*b*-PMLGSLG LB films exhibits two bands at 1736 and 1711 cm^{-1} , corresponding to non-hydrogen-bonded and dimer hydrogen-bonded carboxylic groups, respectively.¹⁷ After annealing, the intensity of the band at 1736 cm^{-1} slightly decreases while that of the band at 1711 cm^{-1} slightly increases (Figure 5), indicating more dimerization of carboxylic groups in the annealed films. A small increase in intensity of the FT-IR band at around 1670 cm^{-1} for the annealed films, visible as a shoulder, also indicates a larger transition dipole coupling between parallel oriented helices.¹⁷ It should be noted that both changes in molecular packing density and the orientation of a certain vibration mode contribute to the variation in its absorption intensity. The increase in molecular packing density in the films would result in an increase in the absorption intensity of a vibration group if the orientation of that group remained unchanged. However, as shown in Figure 5, the absorption intensities of the C=O stretching bands (1738, 1736, and 1711 cm^{-1}) are almost unchanged after annealing, indicating reorientations of the side chain ester and acid groups causing a decrease in IR absorption. Such reorientations could arise from changes in the hydrogen bond interactions of the carboxylic groups as well as the orientations of the main and side chains upon annealing. Due to water removal, accompanied with a molecular rearrangement, the substrate/PLGA and PLGA/PMLGSLG interface roughnesses increased considerably.

It has been found that, due to the transfer-induced orientation effect, in monolayers of PLGA-*b*-PMLGSLGs, the α -helices are oriented azimuthally, forming a smectic C-like phase.²⁶ On the

other hand, the melting point of the partly crystalline stearyl side chain has been reported to be around 20 °C for cast films of PMLGSLG.²⁹ The stearyl side chain of star-shaped poly(γ -stearyl-L-glutamate)s has been found to start to melt at 65 °C and to freely move as a liquid at 107 °C.³⁰ Therefore, upon annealing the PLGA-*b*-PMLGSLG films at 100 °C, the stearyl side chain is fully flexible, acting as a liquid mantle for the PMLGSLG helix cores. Along with the surface area shrinkage during the annealing process, both the smectic C-like order of the helices and the mobility of the side chain mantle seem to favor the increased packing and decreased tilting toward the substrate-normal of the PMLGSLG block helices.

When there is no desorption of the polymer from the substrate surface and no change in the polymer chemical structure upon annealing, the change in surface area can be estimated from the changes in electron density and thickness of the layer by the following relation:

$$\rho_i L_i S_i = \rho_i^{\text{ann}} L_i^{\text{ann}} S_i^{\text{ann}} \quad (2)$$

where ρ_i , L_i , and S_i are the electron density, layer thickness, and surface area of slab i , respectively, and the superscript “ann” indicates the annealed sample. Indeed, the XRR fit parameters (Table 1) show that the changes in surface areas, derived from the changes in electron density and thickness, of the PLGA ($i = 1$) and PMLGSLG ($i = 2$) slabs are in accordance with each other:

$$\frac{S_1^{\text{ann}}}{S_1} = \frac{\rho_1 L_1}{\rho_1^{\text{ann}} L_1^{\text{ann}}} \approx \frac{\rho_2 L_2}{\rho_2^{\text{ann}} L_2^{\text{ann}}} = \frac{S_2^{\text{ann}}}{S_2} \quad (3)$$

Depending on the electron density of the PLGA slab in the original films, the decrease in surface area due to film shrinkage was ca. 14–22%, reaching a maximum electron density of $0.546 \pm 0.003 \times 10^3 \text{ e}^-/\text{nm}^3$ for the PLGA block. This value is about 9% higher than that calculated from the core density of esterified polyglutamates in the solid state ($0.503 \times 10^3 \text{ e}^-/\text{nm}^3$),³¹ attributed to the liquid-crystalline-like order and very densely packed structure in the monolayers of unidirectional aligned helices. Such a high value of electron density, compared with the bulk isotropic state, is not inappropriate, since the density change at an isotropic–smectic transition for rodlike molecules can be rather large ($\sim 10\%$ or more).^{32,33} The electron density of the PMLGSLG slab increased by 6–10%, suggesting a large increase in side chain interdigitation as well as in the orientational order of the rods. The tendency of the side chains to interdigitate more strongly resulting in an increase in electron density and decrease in film thickness upon annealing has been reported for multilayer LB films of PMLGSLG with the helices oriented parallel to the substrate.^{34,35}

To compare with the LB films of PLGA-*b*-PMLGSLGs, we studied a multilayer LB film of PMLGSLG by transmission FT-IR. After annealing, no change in the amide bands was found for the PMLGSLG film, indicating a preserved parallel helix orientation with respect to the substrate. Nevertheless, the same trend for

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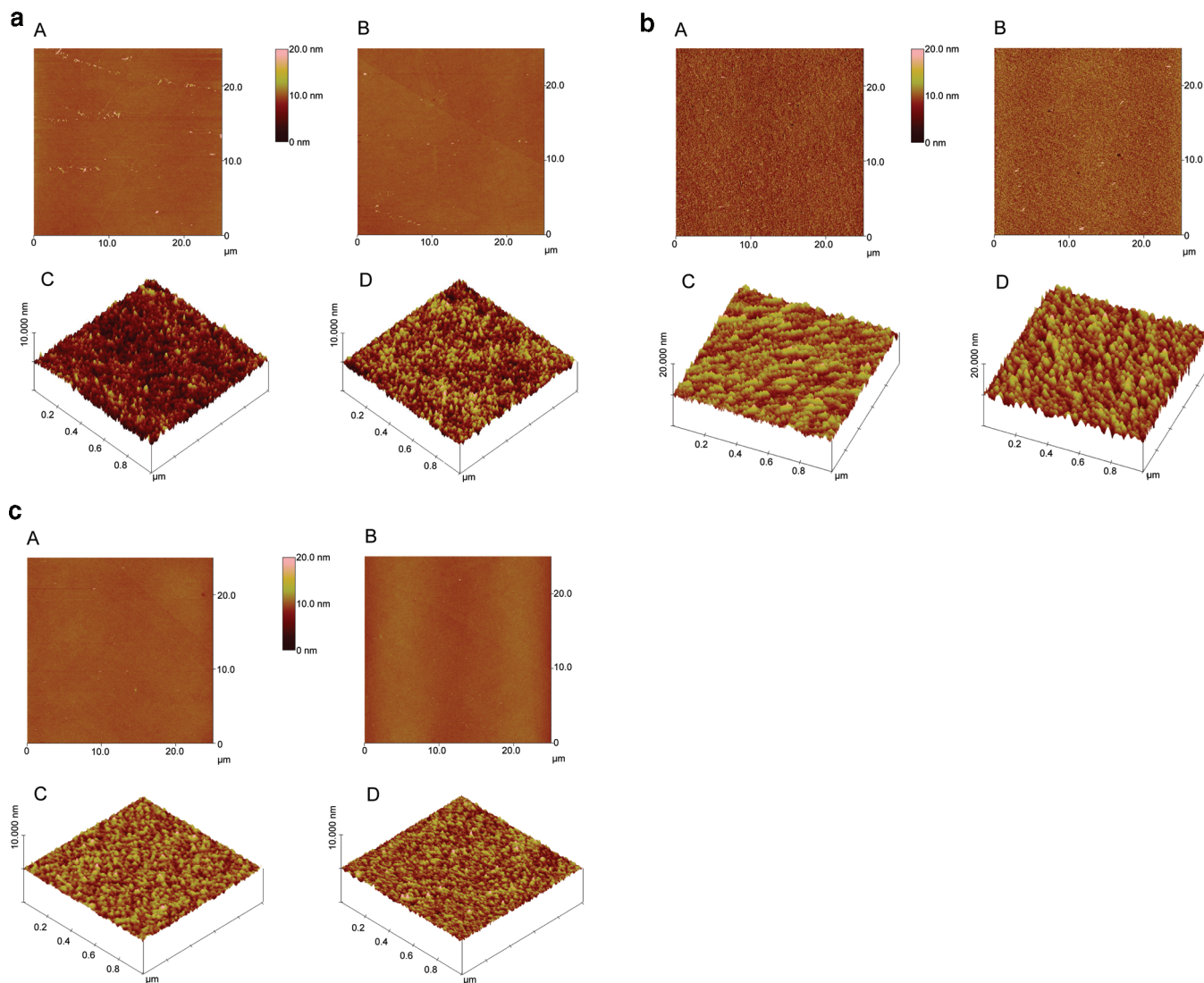


Figure 4. (a) AFM height images of an LB monolayer of CoPo_60_19 transferred onto a silicon substrate at 40 mN/m before (A and C for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) and after (B and D for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) annealing at 100°C for 24 h. Except for some dust particles (white dots in the $25 \times 25 \mu\text{m}^2$ images), the AFM images show homogeneous films. (b) AFM height images of an LB monolayer of CoPo_37_24 transferred onto a silicon substrate at 45 mN/m before (A and C for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) and after (B and D for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) annealing at 100°C for 24 h. (c) AFM height images of an LB monolayer of CoPo_59_82 transferred onto a silicon substrate at 35 mN/m before (A and C for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) and after (B and D for scan areas of $25 \times 25 \mu\text{m}^2$ and $1 \times 1 \mu\text{m}^2$, respectively) annealing at 100°C for 24 h.

change in the methylene stretching vibration bands was observed for both LB films of PLGA-*b*-PMLGSLGs and PMLGSLG: the bands were shifted to higher frequencies and the band absorptions slightly decreased (Figures 6 and 7).

The FT-IR frequencies of the asymmetric and symmetric methylene stretching vibrations are sensitive to the *trans/gauche* conformer ratio of the alkyl chains, shifting to higher wavenumbers with increasing number of *gauche* conformers.^{36,37} Both pressure and temperature affect *trans-gauche* populations.³⁸ For the LB films of PLGA-*b*-PMLGSLGs and PMLGSLG, compressed and transferred at 20°C , the $\nu_a(\text{CH}_2)$ and $\nu_s(\text{CH}_2)$ bands are around $2924\text{--}2925$ and $2854\text{--}2855\text{ cm}^{-1}$, respectively, indicating a substantial number of *gauche* conformers (Figure 6).^{36,37}

As shown in Figure 7, these frequencies correlate decreasingly with the electron density of the PMLGSLG slab. This shows that the *gauche* population decreases, due to enhanced side chain order, with increasing molecular packing of the layer. A similar observation has been reported for a monolayer of poly(γ -methyl-L-glutamate-*ran*- γ -stearyl-L-glutamate) with 50 mol % of stearyl substituents spread at the air–water interface at 20°C .³⁸ At ambient conditions, the *trans/gauche* ratio of the alkyl side chain, in its “unmelted” state, increases with increasing monolayer compression.

Upon annealing, the shift in $\nu_a(\text{CH}_2)$ and $\nu_s(\text{CH}_2)$ to higher frequencies indicates an increase in the *gauche* population, in agreement with the occurrence of a liquidlike phase (Figure 7). The *trans* to *gauche* conversion upon melting allows the alkyl chain to compact itself into tightly coiled conformations in response to the increased packing of the main chain rods.^{39–41}

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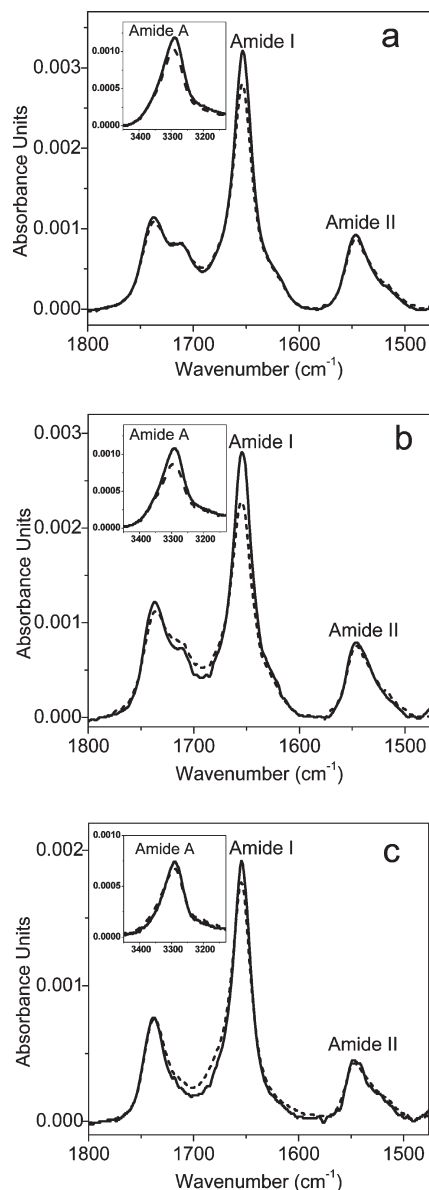


Figure 5. Transmission FT-IR spectra of the LB monolayers of CoPo_60_19 (a), CoPo_37_24 (b), and CoPo_59_82 (c) transferred onto silicon substrates before (solid lines) and after (dashed lines) annealing at 100 °C for 24 h.

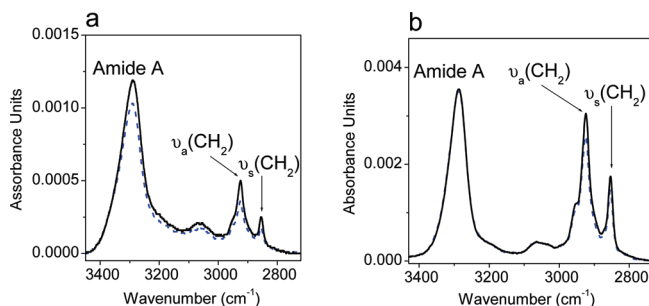


Figure 6. High-frequency transmission FT-IR spectra of an LB monolayer of CoPo_60_19 (a) and a multilayer of PMLGSLG (b) transferred onto silicon substrates before (solid lines) and after (dashed lines) annealing at 100 °C for 24 h.

By coiling, the alkyl side chain can optimally fill a confined space and maximize hydrocarbon density.^{39,40}

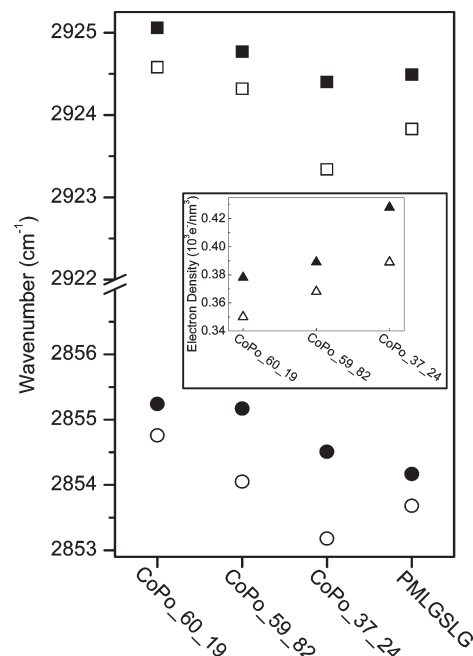


Figure 7. Peak maximum frequencies of the methylene symmetric (circles) and asymmetric (squares) stretching vibrations for the LB monolayers of PLGA-*b*-PMLGSLGs and a multilayer LB film of PMLGSLG. Inset: Electron densities of the PMLGSLG slab in the corresponding PLGA-*b*-PMLGSLG LB films. The open and filled points represent the samples before annealing and after annealing at 100 °C for 24 h, respectively.

The slight decrease in the $\nu_a(\text{CH}_2)$ and $\nu_s(\text{CH}_2)$ absorptions for the films after annealing suggests reorientation of the side chains toward the surface plane.⁴² Under compression on the water surface, the side chains are stretched away from the water surface and this nonequilibrium arrangement is retained in the transferred films. Hence, the orientation change of the side chains upon annealing can be related to their relaxation toward a more uniform distribution in the layer.

Both the decrease in film surface area as a result of considerably increased intermolecular packing of the PLGA block caused by hydrogen bonds between the carboxylic groups upon annealing and the melting of the alkyl side chain into a completely mobile phase accommodate a very dense packing of the PMLGSLG block helices and thereby their reorientation toward the substrate-normal. From a comparison of the annealing effects on the LB films of the PLGA-*b*-PMLGSLG diblock copolymers, it follows that the surface area shrinkage and thereby the change in helix orientation are greater for the diblock copolymers with small block lengths, that is, CoPo_60_19 and CoPo_37_24. Though the trend for an increase in electron density of the slabs is similar for all the diblock copolymers, for CoPo_59_82 there was only a slight increase in thickness and so an insignificant change in helix tilt angle for both blocks. This might be partly due to the preorientation with a large average helix tilt angle in the original film. It might also be that the change in the films upon annealing is dependent on an interplay between the mutual dipolar interactions of the PMLGSLG block helices and the surface constraint caused by the intermolecular interactions between the side chains of the PLGA block segments. For the large hydrophobic block length of CoPo_59_82, the interactions of the off-axis dipole components between the parallel PMLGSLG α -helices might

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tentatively hinder the orientation of the α -helices toward the substrate-normal.¹⁷

Taken together, all the results demonstrate clearly the structural change in the LB monolayers of PLGA-*b*-PMLGSLGs upon annealing. Due to the increased interactions between the carboxylic groups of the PLGA segments upon annealing, the surface shrinkage of the films occurred with an increase in both film thickness and density. As a result of the side chain melting and surface constraint, the PMLGSLG block helices, in a smectic C-like phase, became less tilted toward the substrate-normal and the side chains were reoriented toward the substrate surface with an increase in the *gauche* population.

Conclusions

Annealing, at 100 °C for 24 h, enhanced the helix orientation in double-brush LB monolayers of PLGA-*b*-PMLGSLG diblock copolymers. The annealing process removed the bound water, giving rise to significant interchain interactions between the carboxylic groups of the PLGA segments. In accordance with the surface constraint caused by the shrinkage of the PLGA slab,

the melting of the side chain mantle into a liquidlike state of the PMLGSLG block during annealing favored the helices to be further oriented toward the substrate-normal. This resulted in a more compact uniform film with a decrease in the average helix tilt angle of the PMLGSLG block. For the diblock copolymer with the shorter block lengths, CoPo_60_19 and CoPo_37_24, the postorientation effect was more pronounced and a nearly perpendicular orientation of the PMLGSLG block helices with respect to the substrate was obtained.

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Supporting Information Available: Results on the thermal stability of PLGA-*b*-PMLGSLG and demonstration of the adjustment of the silicon/PLGA interface roughness in the fitting process. This material is available free of charge via the Internet at <http://pubs.acs.org>.